Environmental Health Perspectives
Vol. 57, pp. 249-253, 1984

Analyses of Ethylene Glycol Monoalkyl Ethers and Their Proposed Metabolites in Blood and Urine

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Glycol ethers are known to produce embryotoxic and teratogenic effects in a variety of animal species. In addition, testicular edema and tubular atrophy have been reported. The health effects of this class of compounds are not known in humans, despite the fact that these solvents are widely used in industry.

In order to evaluate potential effects in humans, it is first necessary to estimate exposure in the workplace (environmental monitoring). However, in the case of glycol ethers traditional air monitoring may be ineffective because of the low volatility of these solvents and the possible significant exposure via the skin.

Biological monitoring can be used to estimate glycol ether uptake by all routes of exposure. The compounds can be measured in blood or their metabolites quantitated in urine. These procedures are suggested for measuring 2-methoxyethanol, 2-ethoxyethanol and 2-butoxyethanol in blood. In addition, tentative procedures have been developed to measure the oxidized acidic metabolites, methoxyacetic acid and ethoxyacetic acid in urine as possible indices of exposure. All procedures have detection limits of less than 11 parts per million. These procedures are ready to be validated in workers exposed to these solvents.

Introduction

Historically the alkoxy derivatives of ethylene glycol were thought to be rather benign substances, but experiments in animals have linked 2-methoxyethanol (ME) and 2-ethoxyethanol (EE) or their metabolites methoxyacetic acid (MAA) and ethoxyacetic acid (EAA) to testicular edema and tubular atrophy (*1,2*). Adverse reproductive effects have been noted for ME, EE and 2-butoxyethanol (BE). ME and EE were teratogenic and embryotoxic in pregnant rats and rabbits while BE produced microscopic testicular changes in mice (*3*). In humans, reduced testicular size has been reported in workers exposed to an industrial preparation containing ME (*4*). No other adverse reproductive effects were reported. Others have reported neurologic symptoms, macrocytic anemia and abnormal leukocyte counts in humans exposed to ME (*5*). Increased red cell fragility was noted with BE exposure (*6*). Although there are few reports relating adverse health effects with exposures to ME, EE and BE, these compounds are receiving greater attention because of adverse reproductive effects in both males and females of several species by a variety of exposure routes (*3*). Further human field and epidemiology studies are needed to address this health risk if any in humans.

Ambient environmental sampling does not always assess total worker exposure. The vapor pressures for ME, EE and BE are low. These solvents also are readily absorbed through the skin. Most workers are not exposed to pure glycol ethers but to industrial formulations containing mixtures of glycol ethers and other solvents. In these instances, environmental monitoring should be supplemented with biological monitoring to evaluate not only exposure, but actual uptake of the glycol ethers by the worker. The advantages of biological monitoring are that it accounts for not only environmental concentrations and actual respiratory uptake but also absorption through the skin.

Blood from humans exposed to ME has been shown to contain ME, so one matrix that might be suitable for biological monitoring is blood (*7*). The oxidized acid metabolites of these solvents have been reported in humans and animals: butoxyacetic acid (BAA) in man (*8*) and MAA, EAA, and BAA in animals (*6,9,10*). So

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urinary MAA, EAA, and BAA may also be suitable for biological monitoring.

Most biological monitoring methods must be sensitive with low detection limits. Humans normally are not exposed to the high concentrations of xenobiotics that are often given to experimental animals; therefore, the compounds being sought would be found in lower concentrations than in animals. Theoretically, these low levels are further reduced because the parent compound may be metabolized, conjugated, stored or eliminated. Often the compound is metabolized by more than one route, therefore producing a myriad of possible metabolic products and their conjugates at extremely low concentration.

When an analyst is confronted with the task of quantitating a substance in a blood or urine matrix, the first approach is to isolate the compound from the matrix. The characteristics that make ME, EE and BE ideal for industrial applications make them a challenge for an analytical chemist, that is, their simultaneous lipophilic and hydrophilic properties and low vapor pressures. The hydrophilic nature of glycol ethers prevents their easy isolation from a urine or blood matrix. Many standard gas chromatographic columns will not simultaneously resolve ME, EE and BE, and those that do may not resolve these three glycol ethers from co-extracted substances. Resolution on porous polymer columns are not sufficient, which precludes direct injection of diluted blood. Low vapor pressure for ME, EE and BE precludes headspace analysis. High performance liquid chromatography (HPLC) is not suitable because these compounds and their metabolites do not absorb significantly in the ultraviolet. A gas–liquid chromatographic analysis with flame ionization detector seemed to be the best approach.

Before any analyses could be performed, these solvents and their acid metabolites had to be isolated from the blood and urine matrix. Methylene chloride is an excellent solvent for extraction of ME, EE, BE, MAA and EAA.

ME, EE, BE, MAA and EAA are very soluble in methylene chloride. In addition, methylene chloride is polar, not miscible with water and does not produce a large response in a flame ionization detector.

The purpose of this study is to develop tentative methods for analysis of ME, EE and BE in blood and MAA and EAA in urine from humans. The ability to analyze these substances should permit the evaluation of worker uptake of these solvents and determine whether these methods and analytes are suitable for biological monitoring.

Materials and Methods

Chemicals

Ethoxyacetic acid (98%), and methoxyacetic acid (99%) were obtained from Aldrich Chemical Co. (Milwaukee, WI). 2-Methoxyethanol (99%) was obtained from MCB (Cincinnati, OH). 2-Ethoxyethanol (bp 133–135°C) was obtained from Eastman Kodak (Rochester, NY). 2-Butoxyethanol (99%) was obtained from Mallinckrodt (Paris, KY). Pentafluorobenzylbromide was obtained from Pierce Chemical Company (Rockford, IL). Tetrabutyl-ammonium hydrogen sulfate was obtained from Regis Chemical Company (Morton Grove, IL).

Standard Preparation

Standards containing ME, EE, and BE were prepared in methylene chloride with concentrations ranging from approximately 8 to 1000 μg/mL.

Standards containing MAA and EAA were prepared in methylene chloride with concentrations ranging from approximately 10 to 1000 μg/mL. These acid metabolite standards were then derivatized by using a modified pentafluorobenzylation procedure (*11*) which is summarized here. In a 16 × 150 mm culture tube containing 1 mL of a standard solution is added 1 mL of an aqueous solution which is 0.1 M in tetrabutylammonium hydrogen sulfate and 0.2 M in sodium hydroxide. Then 100 μL of pentafluorobenzyl bromide is added, and the tube sealed and rotated for 2 hr (Roto-torque, Cole Palmer, Chicago, IL). The methylene chloride phase contains the derivatized acids.

Sample Preparation

Blood. A stock solution of the glycol ethers (ME, EE, BE) was mixed with pooled human blood (EDTA anticoagulant). This blood was then diluted with additional whole blood to obtain concentrations ranging from approximately 8 to 1000 μg/g.

Urine. A stock solution of pooled urine containing MAA and EAA was prepared. The stock urine was then diluted with additional urine to obtain concentrations of the acid metabolites ranging from approximately 10 to 1000 μg/mL.

Analytical Procedures

Blood. Blood (1 g), anhydrous sodium sulfate (1 g) and methylene chloride (10 mL) were mixed on a rotator for 2.5 hr. The methylene chloride extract was filtered and reduced in volume to 1 mL. A 3 μL portion was injected into the gas chromatograph equipped with a flame ionization detector (Perkin-Elmer Model 900, Norwalk, CT). The instrument was fitted with a 25 ft. × 1/8 in. stainless steel column packed with 20% Carbowax 20 M on 80/100 mesh Supelcoport (Supelco, Belfonte, PA). Conditions included an injection port temperature of 220°C, column temperature of 200°C, manifold temperature of 250°C, and nitrogen carrier gas of 15 mL/min. Peak area was calculated by using a computing integrator (Spectra Physics Model 4000, San Jose, CA).

Urine. The spiked urine samples were acidified with

HCl to pH 2. A 1 mL aliquot was extracted with 5 mL of methylene chloride three times; the combined methylene chloride extracts were evaporated to 1 mL, and derivatized by using the pentafluorobenzylation procedure described in the standard preparation section. A 5 μL portion of the organic phase was injected into the gas chromatograph (Tracor, Model 560, Austin, TX). The instrument was fitted with a 6 ft. × 1/4 in. glass column packed with 1.95% QF-1, 1.5% OV-17 on 80/100 Supelcoport. Conditions included an injection port temperature of 200°C, a column temperature programmed at 100°C for 2 min, then increasing 10°C/min to 220°C, which was held for 2 min, a detector temperature of 250°C, and the nitrogen carrier flow at 30 mL/min. Peak area was calculated using a computing integrator (Spectra Physics, Model 4100, San Jose, CA).

Results

Blood

The results of the gas chromatographic separation of ME, EE and BE in blood are shown in Figure 1. Figure 1*a* shows a chromatogram of a blank unspiked blood specimen; Figure 1*b* shows the standards in methylene chloride; Figure 1*c* shows a chromatogram containing 100 μg/g of each glycol ether in blood. Approximate retention times for ME, EE and BE are 5.0, 5.4 and 8.0 min, respectively.

Table 1 shows data on detection limits and recoveries for the three glycol ethers in blood. Detection limits for EE and BE are 5.0 and 4.0 μg/g blood, while ME had a slightly larger detection limit of 8.8 μg/g due to its close proximity to the solvent front. Average recovery data ranged from 78% for ME to 84% for EE over a wide concentration range as shown in the table.

Urine

The results of the gas chromatographic separation of the acid metabolites of ME and EE, MAA and EAA in urine are shown in Figure 2. Figure 2*a* shows a chromatogram of urine without added metabolites; Figure 2*b* shows standards of the alkoxyacetic acids in methylene chloride at 100 μg/mL each; Figure 2*c* shows urine containing 100 μg/mL of MAA and EAA. Approximate retention times for MAA and EAA are 4.9 and 5.4 min. Table 2 shows data on detection limits and recoveries for MAA and EAA in urine. Both compounds show baseline resolution. Detection limits for MAA and EAA are 11.4 and 5.0 μg/mL. Recoveries determined over a concentration range of approximately 10 to 1000 μg/mL ranged from 78% for MAA to 91% for EAA. Ranges of

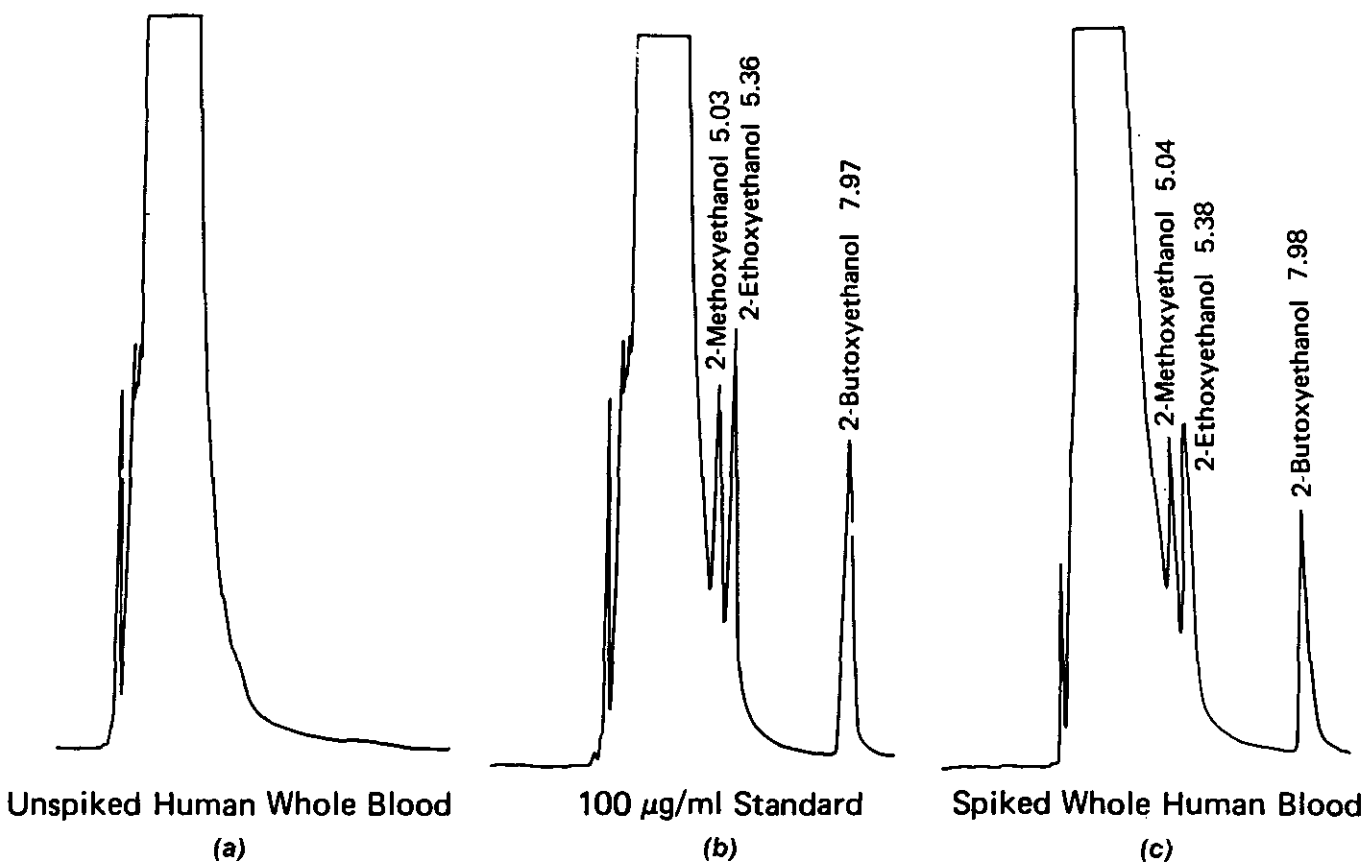

FIGURE 1. Gas chromatographic separation of ME, EE and BE in blood: (*a*) blood with no glycol ethers; (*b*) standard containing 100 μg/mL of each glycol ether; (*c*) blood containing 100 μg/g of each glycol ether.

Table 1. Detection limits and recoveries of 2-methoxyethanol, 2-ethoxyethanol and 2-butoxyethanol in whole blood.

Glycol ether	Detection limit, μg/g	Concentration range in blood, μg/g	Number of extractions	Average recovery, %	RSD	Recovery range, %
2-Methoxyethanol	8.8	88–946	10	78	0.106	63–94
2-Ethoxyethanol	5.0	8.6–895	15	84	0.091	71–99
2-Butoxyethanol	4.0	7.8–879	15	81	0.161	64–105

Table 2. Detection limits and recoveries for methoxyacetic acid and ethoxyacetic acid in urine.

Compound	Detection limit, μg/mL	Concentration range in urine, μg/mL	Number of extractions	Average recovery, %	RSD	Recovery range, %
Methoxyacetic acid	11.4	11.4–1140	15	78	0.17	54–96
Ethoxyacetic acid	5.0	10–1000	15	91	0.14	66–114

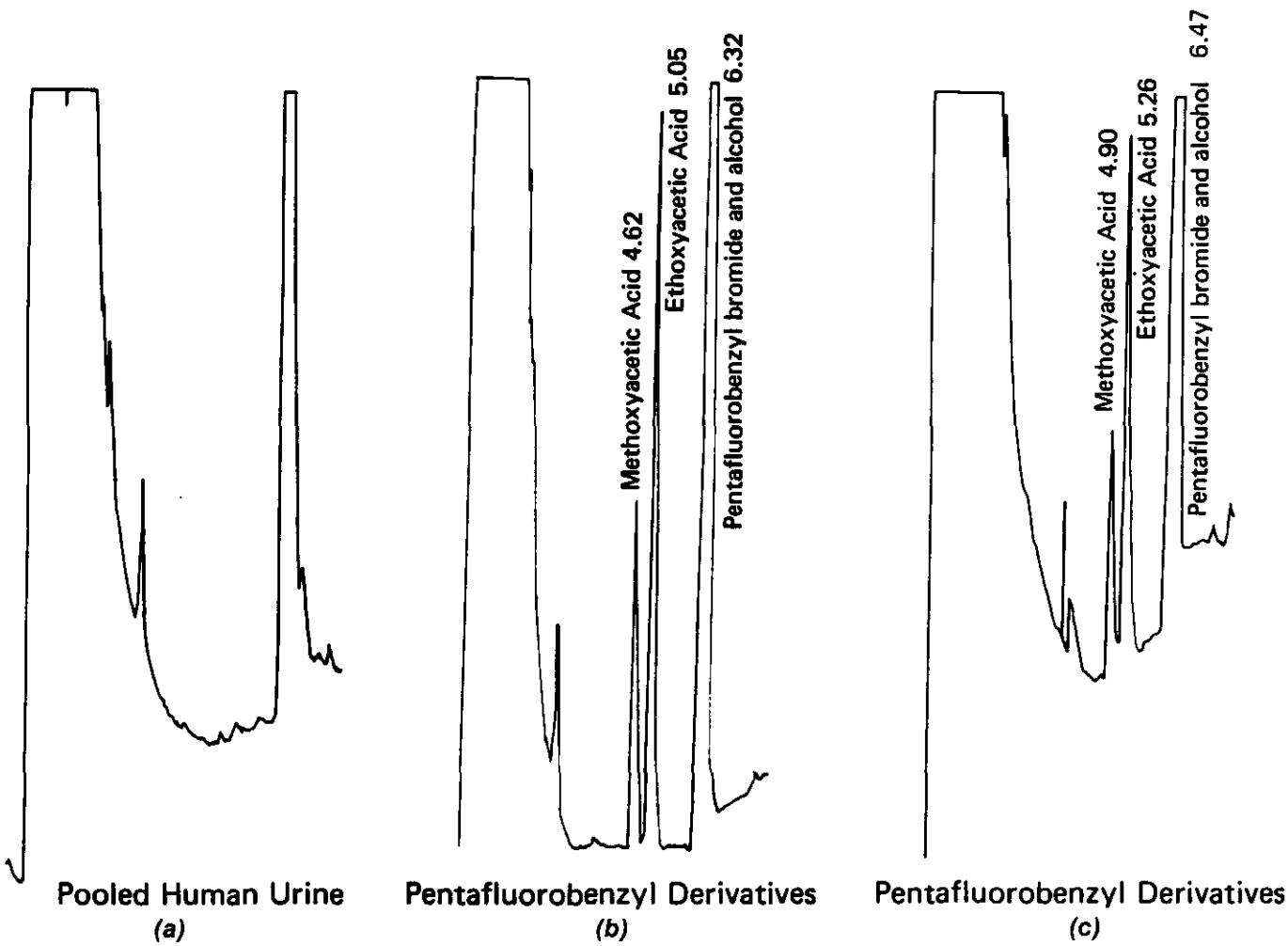

FIGURE 2. Gas chromatographic separation of MAA and EAA in urine: (*a*) urine with no additions; (*b*) standards containing 100 μg/mL each metabolite; (*c*) urine containing 100 μg/mL of each metabolite.

recoveries and relative standard deviations of recoveries are shown in Table 2.

Discussion

The analysis of blood samples as indicators of exposures of workers to ME, EE and BE has not been employed. The pharmacokinetics, metabolism and elimination data in humans is not available. There is one reference where glycol ethers were determined in blood following dermal exposure. Nakaaki, Fukabori and Osamu (7) exposed 12.5 cm^2 of forearm to ME on two human subjects. A rapid rise of ME in blood was reported after 2 hr. The authors reported blood concen-

trations of 106 to 493 μg/mL. The methods used were not detailed; however, a recovery of 66% was reported. The author concluded that whole blood would be an appropriate matrix for monitoring exposures to ME. The method reported here has a wider analytical range, lower detection limits and greater percent recoveries. Therefore, a wider range of exposure at a multitude of time intervals could be monitored.

Perhaps the best approach for biologically monitoring ME, EE, and BE exposures is the quantitation of MAA, EAA and BAA in urine. BE is metabolized to BAA (*6*) in man. Animal studies have strongly suggested that ME is metabolized to MAA in man (*1,9*). There are more analytical techniques and information available relating to analyses of organic acids in urine. Urine samples can be treated in a variety of ways to assure stability of an analyte.

The acids MAA, EAA and BAA can be extracted and derivatized with diazomethane, *N,O*-bis(trimethylsilyl) trifluoroacetamide and bis(trimethylsilyl)acetamide (*11,12*).

However, the pentafluorobenzylbromide was chosen because it was a two-step extractive alkylation technique (*11*). This derivative had been used with some success on butoxyacetic acid. The pentafluorobenzyl derivatives are very electrophilic and would produce a good response in electron capture detectors. This would permit lower detection limits and the possibility of quantitating MAA and EAA at the parts per billion level. However, to extend the range to low parts per billion, further isolation techniques need to be applied to the analyte or its derivative. Sometimes double extraction, each at different pH, is sufficient. Another technique is to treat the sample with XAD resin columns and elute with an appropriate solvent. The most widely used cleanup technique incorporate Florisil and silica gel columns utilizing a variety of nonelectrophilic solvents. The incorporation of some of the abovementioned refinements would permit lower detection limits and may allow monitoring of workers exposed to low concentrations of glycol ethers.

The authors wish to thank D. Gayle DeBord for her extensive bibliography on alkoxyethanols compiled while submitting a research proposal for the Ph.D.

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